REMARKS

Applicants thank the Examiner for the thorough review and examination of this application. In response to the Office Action, the specification and certain of the claims have been amended, as is discussed in more detail immediately below and in the sections that follow.

The extensive amendments to claim 1 have been made in part as a response to various of the objections and rejections as noted in the discussion. Sections of claim 1 have been rearranged somewhat into a more logical order. Certain changes to the claim language are intended merely to add clarity.

Support for the various amendments and new claims are discussed in the sections below. Most of the new claims find support in the original claims. New claim 18 supported at least by original (PCT) claim 4. Claim 19 is supported at least by original claim 5 and claims 20-22 find support at least in original claim 6.

New independent claim 24 has been added to remove the "chromosome-derived" language present in claim 1. Otherwise, it is identical to amended claim 1. Support for this claim can be found, inter alia, at page 5, lines 23-25 which read: "It is also very easy to determine the DNA concentration and hence the copy number of the nucleotide sequence per volume"

As a result of the addition of eight new claims, the claims now under examination are claims 1-24, of which claims 1 and 24 are independent. No new matter is added by the present amendments and new claims, and their entry is respectfully requested. In view of the amendments and remarks below, it is believed that the present application is in condition for allowance. Applicants therefore request early notification of such allowance.

I. Objections to Specification

The specification was objected to for several reasons, each of which has been corrected by the above amendments.

At page 7, before description oft the drawings, the following line was inserted: "Brief Description of the Drawings". The references to "circles" in the drawings (Figs. 1-4 at page 7) have been amended to read "diamonds."

All trademarks that Applicants recognize as such have been amended to be in capital letters followed by either the symbol ™ or ®.

II. Claim Objections (Item 4)

Claim 1 was objected to because the grammar was said to be unclear based on allegedly redundant use of the word “relative”.

Applicants’ Response

The following remarks relate both to this objection and to the indefiniteness rejections set forth in Items 5 and 7 of the Action.

The objected to language has been corrected in amended claim 1. Claims 1 and 2 have been amended to be more specific in distinguishing “CN”, “relative CN” and “absolute CN”. The amendment to claim 1 remedies the objection in Item 4.

Claim 1 now reads in relevant part:

…to obtain the relative CN of NucSeqI with respect to NucSeqII by the formula:

$$\text{relative CN} = \frac{\text{Conc-I}_{SCI}}{\text{Conc-II}_{SCII}}$$

wherein, in said formula,

(i) “relative CN” is the ratio of the CN of NucSeqI relative to the CN of NucSeqII in the sample;

New claim 18 and amended claim 2 read:

18. A method according to claim 1, wherein an **absolute** number of copies of NucSeqII per cell is known.

2. A method for determining the **absolute** CN of a nucleotide sequence NucSeqI in a sample comprising:

(a) determining the **relative** CN using the method of claim 18, and

(b) multiplying the **relative** CN by the **absolute** CN of NucSeqII per cell.

III. Rejections under 35 USC § 112/second paragraph

A. First Rejection for Indefiniteness (Item 5a)[1]

Claims 1-16 were rejected as indefinite because the term “CN” was allegedly used to stand for both the copy number of NucSeqI in line 1 (claim 1) and as the relative copy number of NucSeqI (claim 1, para. (d)(i)). Thus it was considered unclear and indefinite as to what “CN” represents.

Applicants’ Response

Claims 1 and 2 (and new claims 17 and 18) have been amended to better distinguish "CN," "relative CN" and "absolute CN" (claim 1, 2, 17and 18). Applicants believe that on this basis (and in view of the remarks below), this basis for rejection may be withdrawn.

B. Second Rejection for Indefiniteness (Item 5b)

A second basis for alleged indefiniteness was applied to claim 1 (lines 17 and 18), because it was `said to be unclear how the ratio of the concentration of NucSeqI' and NucSeqI' related to the method of determining a copy number or relative copy number.

Applicants' Response

The ratio of the concentration of NucSeqI' to the concentration of NucSeqII' in claim 1 relates first to the generation of standard curves from which the concentration of NucSeqI and NucSeqII can be determined. More importantly, the generated standard curves are known to be based on the same concentrations of the two sequences, because NucSeqI' and NucSeqII" are localized on a single vector (as noted in claim 1). This permits accurate determination of the concentration of NucSeqI relative to the concentration of NucSeqII in the test sample.

The following "example" may help clarify the claim language. If a single copy of NucSeqI' and of NucSeqII' are present a single vector, it is understood that their concentrations will be equal within a sample. Since NucSeqI and NucSeqII are determined based on the standard curves constructed from multiple dilutions of NucSeqI' and NucSeqII', respectively, uncertainty in the measurement is reduced as compared to a situation in which the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' is not so absolutely known.

Furthermore, use of "relative" values makes the claimed method more robust. For example, if an error, such as a pipetting error, were to occur in the dilution of the nucleic acid (single vector) that carries NucSeqI' and NucSeqII', the concentrations of NucSeqI and NucSeqII that are determined from the standard curves will be incorrect. However, the calculated relative CN and absolute CN will be correct.

Support for the phrase "ratio of concentration of NucSeqI' and the "concentration of NucSeqII'" can be found in the specification at page 3 lines 30-34, and page 5 lines 33-38.

C. Third Rejection for Indefiniteness (Item 6)

[1] "Item numbers" correspond to the paragraph/section numbering in the Office Action. 5s and 5b refer to separate paragraphs under Item 5, though these letter designations are not found in the Action.

Claims 1-16 were said to be "incomplete" due to alleged omission of essential steps, creating a "gap" between the steps. According to the Office, the omitted steps are "constructing the standard curves SC_I and SC_{II}.

The Office Action noted that "missing" items included:

(i) parameters of NucSeqI' and NucSeqII' that are known, and

(ii) what measurements are made on NucSeqI' and NucSeqII'.

D. Fourth Rejection for Indefiniteness (Item 7)

Claims 1, 3-5, 8-9, and 11-16 were said to be "incomplete" because they allegedly omitted essential steps, creating a "gap" between the steps. The omitted steps are "determining the copy number of a first nucleotide sequence" as recited in the preamble of claim 1. The Office contends that, while Claim 1 does recite a step for determining the relative copy number in claim 1(d), the twelve rejected claims do not recite determining an absolute copy number.

Applicants' Response

The amendments to claim 1 and 2, along with the introduction of new claims 17 and 18, are discussed above. Applicants believe that in view of the amended language and the new claims, the above two grounds for rejection may be withdrawn.

E. Fifth Rejection for Indefiniteness (Item 8)

Claims 2, 6, 7, 9, 10, 13, 14, and 16 recite the limitation "cell" (claim 2, line 3) without a insufficient antecedent basis for this limitation.

Applicants' Response

Applicants disagree with this rejection since the introduction of "cell" in claim 2 does not create any ambiguity in the claim language, and is not preceded by "the" or "said." Nevertheless, the addition of new claim 17 introduces the term "cell" for which support can be found throughout the specification, for example, page 5, lines 1-17. As noted above, new claim 18 supported at least by PCT claim 4, claim 19 is supported at least by PCT claim 5 and claims 20-22 find support at least in PCT claim 6.

F. Sixth Rejection for Indefiniteness (Item 9)

Claims 7-9 were rejected because, according to the Office, the language "NucSeqI and NucSeqI' are the same" allegedly conflicts with the language in base claim 1 which reads "NucSeqI' corresponds to NucSeqI." The Action notes that the specification defines "corresponding" as where one sequence is complementary to another sequence (citing page 3 line

35 through page 4 line 8). Thus, the Office interprets NucSeqI' in claim 1 as being complementary to NucSeqI. Hence, the two cannot be the <u>same</u> sequence. This allegedly renders claims 7-9 unclear and indefinite.

<u>Applicants' Response</u>

Applicants wish to clarify the Examiner's confusion about an apparent conflict between the terms in Claim 1 as they related to rejected Claims 7-9. The answer lies in careful review of the description/definition at page 3 line 35 to page 4, line 5) to which the Action referred. A direct quotation of the specification is:

> …With respect to the term "corresponding" as used in the present invention in conjunction with nucleotide sequences, this is intended to mean that the nucleotide sequences I and I' (and II and II'), or more specifically the nucleotide <u>sequence of one</u> and the <u>complementary sequence of the other</u>, are capable of hybridizing under stringent conditions…

(emphasis added)

It should be evident that the sequences of NucSeqI and NucSeqI' must have a relatively high degree of homology so that the NucSeqI hybridizes under stringent conditions with the ***<u>complementary</u>*** **<u>sequence of</u>** NucSeqI'. Thus, if NucSeqI "corresponds to" NucSeqI' it is "homologous to" NucSeqI' to the extent required by stringent hybridization (of NucSeqI to "the complement of" NucSeqI'. This "correspondence" or "homology" would obviously include the possibility that NucSeqI and NucSeqI' are identical (claims 7-9) without being in conflict with claim 1. In view of the foregoing clarification, Applicants believe that this rejection should be withdrawn.

G. <u>Seventh Rejection for Indefiniteness (Item 10)</u>

Claims 10-16 were rejected for the same reasons given above in rejecting claims 7-9.

<u>Applicants' Response</u>

The foregoing remarks regarding claimss7-9 apply similarly to the rejection of claims 10-16 in the context of the relationship of NucSeqII and NucSeqII'.

<u>IV. Rejections for Lack of Enablement under 35 USC § 112, First Paragraph *(Items 11-13)*</u>

The Office Action has rejected claims 1-16 as lacking an enabling disclosure for reasons set forth below. These points are discussed below as are Applicants responses to them.

At the outset, however, Applicants believe that the Office has not met its burden required by the law in setting forth a *prima facie* rejection for lack of enablement. It respectfully is submitted that when the proper test for enablement is applied to the pending claims, considered in light of the respective burdens upon an applicant and the Patent Office in establishing and

responding to a *prima facie* case of nonenablement, the claims should be found to be enabled and allowable.

A. Legal Test for Enablement:

The enablement requirement of 35 U.S.C. § 112, first paragraph, ensures that one skilled in the art will be able to make and use a claimed invention. *Raytheon Co. v. Roper Corp.*, 220 USPQ 592, 599 (Fed. Cir. 1983). That some experimentation may be required does not preclude a finding of enablement so long as the amount of experimentation is not unduly extensive. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 220 USPQ 303, 316 (Fed. Cir. 1983). Furthermore, there is "no magical relation between the number of representative examples and the breath of the claims; the number and variety of examples are irrelevant if the disclosure is 'enabling' and sets forth the 'best mode contemplated.'" *In re Borkowski*, 164 USPQ 642, 646 (CCPA 1970). A specification, in fact, need not contain a single working example. *Id.* 164 USPQ at 645.

The Office has the burden of establishing a lack of enablement. *In re Hogan*, 194 USPQ 527, 539 (CCPA 1977). Factors to be considered in determining whether pending claims would require undue experimentation have been articulated by the Court of Appeals for the Federal Circuit in *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). They include:

> (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

In this case, the Office does not appear to have undertaken, and has not provided to the Applicants, a legally sufficient *Wands* analysis and has not set forth evidence to demonstrate that one skilled in the art would find the specification nonenabling in light of its disclosure and exemplification. Much of the analysis appears to constitute the Examiner's personal views of the disclosure and its enabling support (or lack thereof) for the claims. Thus, the Action has not provided Applicants with an assessment of enablement under the "standard of reasonableness" to which they are entitled, given the nature of the invention and the state of the art.

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. [citations omitted] The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed…

In re Jackson, 217 USPQ 804, 807 (Bd. App. 1982, cited with approval in *Wands*, 8 USPQ2d at 1404).

Even when "unpredictability" in a field (such as chemistry) may create reasonable doubt as to the accuracy of a broad statement supporting enablement, and even when the statement is, on its face, contrary to generally accepted scientific principles, the Court of Customs and Patent Appeals (predecessor to the Federal Circuit), has clearly articulated that

> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with a contested statement.

In re Marzocchi, 169 USPQ 367, 369 (CCPA 1967).

As discussed below, the specification does provide support for the methods as presently claimed, allowing a person skilled in the art to practice the claimed invention to its full scope without undue experimentation or further inventive effort.

B. Specific Grounds of Rejection and Applicants' Responses

1. Spectrophotometric measurement

Claims 1-16 allegedly failed to comply with the enablement requirement. These claims are said to broadly recite a method of determining a copy number of a nucleotide sequence spectrophotometrically which includes colorimetric, fluorescent, and luminescent measurements. However, the Action notes that the specification only discloses fluorescent measurements with oligonucleotides that are labeled with a fluorophore.

Applicants Response *(regarding Spectrophotometric Monitoring)*

Claim 1 has been amended to read "monitored by fluorescence" thereby rendering this aspect of the rejection moot. The Action indeed confirms that the specification supports this language. (citing Example 1, page 8 lines 5-9, 14-16).

2. Relative vs. Absolute Copy Number/Use of Probes

The Office accepts that, as broadly claimed, these rejected claims do recite a method that determines a ratio which is not a relative copy number (CN). Claim 2 and claims dependent therefrom also are said to recite a method which uses this ratio but the resulting determination is not an absolute CN, as broadly claimed. The Examiner asserts that the ratio and subsequent uses of the ratio would be accurate only under limited conditions and not as broadly claimed.

Of the two Examples in the disclosure, Example 2 allegedly does not provide direction on how to determine a CN (or relative CN). Rather, the Example is said to describe how to use copy numbers of a certain cellular oligonucleotide that decreases in cells from aging humans. Example 1 allegedly also lacks directions as to what is being measured and what would be done

with those measurements. Thus, the Office asserts that Example 1 does not provide sufficient direction on how to obtain a CN or a relative CN. Example 1 also refers to the use of "probes" in the invention. However the claims lack this element (probes) and do not include a step that uses the probes. Thus the claims lacking such a "probes" limitation are considered not to be enabled.

The Office concluded that for the above reasons, undue experimentation would be needed to use the invention as it is broadly claimed. The Action further asserts that the claims lack enablement with respect to both absolute copy number and relative copy number.

The Office Action provides a detailed discussion as to why the claims to the extent they recite "relative CN" lack enablement. The ratio, claimed to be a relative CN, is determined by dividing Conc-I_{SCI} by Conc-II_{SCII} (equation for "CN" in claim 1, part (d)). Conc-I_{SCI} is stated to be the copy number of the first sequence, NucSeqI; and Conc-II_{SCII} is claimed to be the copy number of the second sequence, NucSeqI.

Applicants' Response

With respect to the absence of "probe" language, Claim 1 has been amended to include "probes." This amendment is supported at least by Example 1 (page 8 lines 15-27).

Applicants believe that their other amendments to claim 1 (plus presentation of new claims) overcome this rejection, since they provide language that is necessary and appropriate for a method directed to determine relative and absolute CN. It should be clear from the above that claim 1 (and claims dependent therefrom that are not directed to "transformation" of "relative" to "absolute" CN), set forth a fully enabled method for determining relative CN. Amended claim 2 and claims dependent therefrom set forth an enabled method for determining absolute CN.

There should be no question that the techniques of RT-PCR, generation of primers and probes, and the conditions for running RT-PCR are conventional and well-known to those skilled in the art. Determining the concentration of a nucleotide sequence using a standard curve is also routine. Claims are not expected to recite such factors that are well-known and would be considered obvious to one of ordinary skill in the art to whom the specification and claims are directed (*In re Skrivan*, 166 U.S.P.Q. 85 (C.C.P.A. 1970). As stated in *In re Chelowsky*, 108 USPQ 321, 324 (C.C.P.A. 1956) quoted w/ approval in *In re Folkers*, 145 USPQ 390, 394 (C.C.P.A. 1965):

> ... the applicant 'may begin at the point where his invention begins, and describe what he has made that is new and what is replaced of the old. That which is common and well-known is as if it were written out in the patent***'

Thus, a person of ordinary skill armed with the present specification will be able to use the invention as currently claimed without undue experimentation to determine relative and absolute CN.

3. The Office's Discussion of Colorimetric Measurements (Item 12)

The Action alleges that Conc-I_{SCi} and Conc-II_{SCii} are copy numbers of NucSeqI and NucSeqII, respectively. The Action goes on to state that, for colorimetric measurements, Conc-I_{SCI} can be biased (wrong) due to the calculation of Conc-I_{SCI} by applying the colorimetric measurement of NucSeqI to a standard curve of NucSeqI'. As viewed by the Examiner, the specification discloses that NucSeqI' is a sequence which is complementary to part or all (50% to 100%) of NucSeqI. Because of this complementarity and the fact that NucSeqI' can be of a different length than NucSeqI; the standard curve of NucSeqI' allegedly does not permit determining an accurate copy number for NucSeqI. An accurate copy number (of NucSeqI) will only be possible in the limited circumstance where the extinction coefficient of NucSeqI is equal to the extinction coefficient of NucSeqI'. In other words, it would be required that same concentration of NucSeqI and NucSeqI' yield the same colorimetric measurement.

The correct calculation of Conc-II_{SCII} is subject to a parallel analysis, where Conc-II_{SCII} is calculated by applying the measurement of NucSeqII to the standard curve of NucSeqII'.

The Action further notes that any bias in measurement of Conc-II_{SCI} is independent of any bias in measurement of Conc-II_{SCII}. (*Applicants believe that the examiner meant to say* Conc-I_{SCII} *here)*. Since the biases need not be equal, they will not necessarily cancel each other out in the calculation of the ratio of Conc-I_{SCI} to Conc-II_{SCII} (formula in claim 1). Thus the ratio too can be biased and is therefore not a relative CN for a first nucleotide sequence, NucSeqI.

In regards to NucSeqI' being a different length than NucSeqI, the Action refers to the Zhang *et al.* reference (discussed below in the context of prior art rejections):

> "...it was known that the difference in the size of PCR products affects their optical density *[colorimetric measurement]*. By using K_s *[a size ratio constant]*, the potential error in calculating the number of mRNA copies has been corrected."

(citing page 774, 2nd column, 2nd paragraph; emphasis refers to examiner's comments inserted into quotation).

Applicants' Response

Respectfully, Applicants believe that the Examiner has made an incorrect assumption in the last paragraph above setting forth the rejection. It is wrong to conclude that Conc-I_{SCi} and Conc-II_{SCii} are claimed to be the <u>copy numbers</u> of NucSeqI and NucSeqII, respectively. Claim 1 defines Conc-I_{SCi} and Conc-II_{SCii} to be the <u>concentration</u> of NucSeqI and NucSeqII, respectively, using the standard curves SC_I and SC_{II}, respectively. Basis is found on page 2 lines 2-5. Thus, the Office's statement that Conc-I_{SCI} can be biased and the reasons for this conclusion are not correct.

Moreover, "colorimetric" measurements do not appear in claim 1 (see discussion of Item 11 above.). It is therefore believed that this basis for rejection (Item 12) is no longer applicable to the amended/new claims.

4. <u>The Office's Discussion of Fluorescence Measurements (Item 13)</u>

The Action points out that, for fluorescent measurements, Ginzinger (discussed below in connection with prior art rejections) teaches probes with fluorescent labels which function as follows:

(a) a probe with a quencher and fluorophore where the quencher is cleaved,
(b) a probe with quencher and fluorophore which separate, or
(c) a probe and a dye which binds only to the hybridized probe (Figure 2 of the reference).

Example 1 of the specification <u>**does**</u> present a probe with a quencher and fluorophore but allegedly does not disclose how this probe functions. This leads to a conclusion that the specification fails to disclose how biases in Conc-I_{SCI} and Conc-II_{SCII}, are corrected colorimetrically or how fluorescence measurements are determined.

The Action alleges that removal of any biases would be necessary to calculate a **relative CN** or an **absolute CN**. Due to the alleged (1) bias in measurements and in calculations of claims 1-16 and/or (2) absence of the essential labeled probe element with the correct function, the claimed invention is not enabled. The Action concluded that one of skill in the art would not know how to use the disclosed invention to determine a absolute CN number or a relative CN of a first nucleotide sequence.

<u>Applicants' Response</u>

Applicants disagree with the foregoing analysis and conclusions. Use of fluorescently labeled probes in RT-PCR methods, as disclosed in Ginzinger, is routine in the art. Therefore, the skilled artisan knows what probes are available and how they work. These aspects are not Applicants' invention, but are firmly within the prior art. In addition, the skilled artisan would

(a) appreciate that any probe known to be useful for RT-PCR may be used in the present methods, irrespective of exactly how it works, and (b) know how to use it.

To summarize, and reiterate, Applicants believe that the Office has not made out an adequate and legally supported *prima facie* rejection for lack of enablement under *In re Wands* for most of the grounds set forth . On that basis alone, it would be proper to withdraw most of the rejections. Notwithstanding this view, Applicants believe that their amendments, new claims and remarks above are sufficient to clarify aspects of the invention as claimed and to overcome any remaining grounds for rejection based on lack of enablement that may have been proper. Thus, it would now be proper to withdraw this ground for rejection.

V. PRIOR ART REJECTIONS

A. Rejections under 35 USC § 102(b) – Anticipation *(Item 14)*

The Office prefaces its art rejections with the following statement, referring to the rejections under §112 discussed above.

> *Due to the claim rejections given above, a distinction cannot be made between the claimed invention and prior art, as given in the following rejection.*

Applicants cannot discern whether this statement refers to the lack of enablement rejection, the indefiniteness rejections, or both. It is hoped that the above amendments and remarks, as well as the discussion below of the cited documents in relation to the claimed invention, are sufficient to assist the Examiner in making the necessary distinctions.

Claims 1-3 and 6 were rejected under 35 U.S.C. 102(b) as being anticipated by Ginzinger *et al., Exper Hematol 30*:503-512 (2002). (This reference properly should have been cited as "Ginzinger" since he is the sole author). The document is referred to below as "Ginzinger".

Ginzinger allegedly teach methods comprising the elements of claims 1-3 and 6. Ginzinger is said to teach additional elements of fluorescent detection, probes with fluorescent labels, and standard curve construction which result in methods that determine an absolute copy number and a relative copy number.

Claim 1

As characterized by the Office, Ginzinger teaches a method of determining a ratio of a first nucleotide sequence comprising the steps of:

(1) adding to the sample nucleotides, primers, polymerase and optionally, any additional reagents, required for amplification (citing the entire reference, especially Figs. 1 and 2);

(2) performing one or more amplification cycles to amplify the target DNA (or mRNA) wherein the sample comprises a chromosome-derived second nucleotide sequence and standard DNA. The amplification steps carried out are:

(a) target DNA is amplified,

(b) standard DNA is amplified,

(c) complements of target DNA and standard DNA are amplified (citing p. 508, 1st column

> "The unknown samples can simply be quantified by deriving the value from a standard curve generated with known samples from any of the three sources (Figure 2)"

and the legend of Figure 4 (p. 510):

> "…with about 5 or 6 bases of the 3 end of one primer being complementary to the adjacent exon…

(that the Examiner notes is "target DNA"); and

(d) standard DNA is present in a control sample and is amplified at multiple dilutions, wherein amplification of the target DNA and standard DNA at multiple dilutions results in the generation of standard curves such that a relative copy number can be determined from the fluorescent spectrophotometric measurements applied to standard curves, and wherein the amplification reaction is performed in a single container (citing to p. 504, last sentence "with up to three different PCR reactions in a single tube") and monitored spectrophotometrically by fluorescence during amplification. Target DNA and standard DNA are localized on a single vector (citing the entire reference, especially Figs. 1 and 2).

Applicants' Response

Claim 1

Ginzinger's definition of relative quantitation appears at page 507, left col, paragraph 3): "relative quantitation compares within a sample the gene of interest to control gene(s)". Quantitation (relative to the control genes) is performed by subtracting the cycle threshold, or "Ct," of the control gene from the Ct of the gene of interest, and the resulting difference in cycle number (ΔCt) is the exponent, base 2 (due to the doubling function of PCR), representing the "fold difference" of template for these two genes.

In the present claims, the concentration of NucSeqII *per se* is determined. NucSeqII is not a selected housekeeping gene that serves as a control gene by subtracting the Ct of the control gene from the Ct of the gene of interest, to yield the difference in cycle number. In fact,

Ginzinger does not disclose a method for determining relative copy number; use of ΔCt is for the sole purpose of controlling for (correcting) *interassay variability*. That is, expression is expressed in relation to that of a "constant" control gene, *e.g.*, a gene which is known to exist as two copies per cell. In this way, Ginzinger determines an approximate copy number (page 508, left col, first para), emphasis added.

Furthermore, in the present claims, NucSeqI' and NucSeqII' must be localized on a single vector. In contrast , the "control gene" used by Ginzinger is located on a chromosome and the standard curve of "knowns" may be generated using a plasmid to carry the gene of interest.

Notably, Ginzinger does not disclose any of the following elements of claim 1;

(i) a single vector comprising two or more different NucSeqI' sequences;

(ii) a third nucleotide sequence I' and/or a fourth nucleotide sequence II', wherein the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' is known; and

(iii) a formula to determine the relative CN (and absolute CN as recited in the relevant dependent claims).

Claim 2

Ginzinger's definition of standard curve quantitation appears at page 507, left col, para. 4): "Standard-curve quantitation has also been termed "absolute quantitation." However, this is a misnomer. A more appropriate term for this method would be "standard-curve quantitation," as a standard curve of "knowns" is used to quantify the "unknowns" of interest. The reliance of Ginzinger's method on a set of knowns is the reason it cannot be "absolute." For generating the standard curve, standards can be selected as those for Q-RT-PCR, but the endogenous genes can be different for the relative quantitation method. To be used correctly, each method has to be tested with a standard curve to determine the presence of PCR inhibitors.

Claim 2, as presently amended, is directed to determining an absolute copy number by multiplying the determined relative CN by the absolute number of copies of NucSeqII per cell. Despite the fact that Ginzinger discloses use of a control gene of which there are two copies in each cell (page 509, right col, last two lines), that is not the same as disclosing the possibility of determining an absolute copy number (see page 508, left column, 1st paragraph, where Ginzinger states: : "… it is possible to approximate the number of copies of a template in an unknown sample, although **not in terms of absolute copy number**" (emphasis added).

Claim 3

Instant Claim 3 is directed to a method for determining the relative copy number of at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI sequences, localized on a single vector. Although Ginzinger discloses use of a standard curve to determine copy numbers, whereby NucSeqI' might be localized on a plasmid (page 508, left col, 1st paragraph), this document also teaches determining multiple NucSeqI (up to three PCR reactions in a single tube; page 504, last sentence). However, Ginzinger does not disclose two or more different NucSeqI' localized on a single vector.

Claim 6

Instant Claim 6 is directed to a method to determine the absolute copy number of at least two different NucSeqI' sequences used for measuring a corresponding number of different NucSeqI sequences, localized on a single vector. As is described for claim 3 above, Ginzinger does not disclose two or more different NucSeqI' localized on a single vector.

In summary, for the reasons discussed above, Applicants disagree with the Office's position that Ginzinger anticipates present claims, particularly in view of the amended claim language. Therefore, Applicants respectfully request that this rejection under §102(b) be withdrawn.

B. Rejections Under 35 USC § 103(a) – Obviousness *(Item 15)*

Here again, the Office prefaces its rejection with the same statement quoted above:

> *Due to the claim rejections given above, a distinction cannot be made between the claimed invention and prior art, as given in the following rejection.*

Claims 1-3 and 6 were rejected as being obvious over J. Zhang *et al.* , *Biochem J. 321*:769-775 (1997) (referred to herein as "Zhang").

Zhang allegedly teaches methods comprising all the elements of claims 1-3 and 6, though **without teaching** a **method of constructing a standard curve with measurements of a sequence complementary to a standard sequence.** But Zhang is said to teach a method comprising additional elements not found in the claims 1-3 and 6. Zhang teaches additional elements of correction factors and proper standard curve construction which result in methods that, per the Office, do determine an absolute CN and relative CN.

Claim 1

As characterized by the Office, Zhang teaches a method of determining a ratio of a first nucleotide sequence comprising the steps of:

(1) adding to the sample nucleotides, primers, polymerase and optionally, any additional reagents, required for amplification (citing entire reference, especially p. 771 and sections titled *Quantitative RT-PCR using various amounts of total RNA* and *Quantitative RT-PCR using various numbers of PCR cycles*);

(2) performing one or more amplification cycles to amplify the target RNA; wherein the sample comprises a chromosome-derived second nucleotide sequence, standard RNA. The following amplification steps are then carried out

   (a) target RNA is amplified,
   (b) standard RNA is amplified,
   (c) complements of target RNA and standard RNA are amplified *(citing the same two sections noted under (1), above)*; and
   (d) standard RNA is present in a control sample and is amplified at multiple dilutions, wherein amplification of the target RNA and standard RNA at multiple dilutions results in the generation of standard curves such that a relative CN can be determined from the spectrophotometric measurements applied to standard curves, and wherein the amplification reaction is performed in a single container (*i.e.*, the same tube) *(citing p. 770, 1st para, last sentence)* and monitored spectrophotometrically during amplification, and target RNA and standard RNA are localized on a single vector (*citing entire reference, especially Fig. 1*);

Zhang is alleged to further teach a method of determining a relative copy number of a nucleotide sequence, notably as follows (citing to page 772):

$$\mathbf{D_t/D_s = (M_tS_t)/(M_sS_t)} \qquad \mathbf{[1]}$$

which is equivalent to:

$$\mathbf{M_t/M_s= (D_tS_s)/(D_sS_t)\ =\ relative\ copy\ number} \qquad \mathbf{[1a]}$$

wherein

M_t is the [copy] number of **m**olecules of **t**arget RNA,
M_s is the [copy] number of **m**olecules of **s**tandard RNA,
D_t is the optical **d**ensity of the **t**arget RNA,
D_s is the optical **d**ensity of the **s**tandard RNA,
S_t is the **s**ize of **t**arget RNA, and
S_s is the **s**ize of **s**tandard RNA,

Claim 2

Zhang is said to teach a method wherein an absolute copy number is determined, per Equation 4 below (citing to page 772):

$$\mathbf{M_m = (M_sK_s)\ X_e} \qquad \mathbf{[4]}$$

wherein

M_m is calculated and is the number of **m**RNA copies in 1 ng of total RNA (copy number/ng),

M_s is known and is the [copy] number of molecules of **s**tandard RNA,

K_s is known, and is the **s**ize ratio constant (the ratio of the size of the standard RNA fragment to that of the target RNA,

X_e is determined from a prior standard curve prepared from known amounts of total RNA versus the ratio of optical densities of target RNA to standard RNA.

Claim 3

Zhang is said to teach a method wherein at least two different sequences used for measuring a corresponding number of different sequences are localized on a single vector (citing entire reference, especially examples for α, β and γ fibrinogen genes, and Fig. 1 where standard RNA was synthesized from reverse-transcribed RNA with primers 1, 3 and 5).

Claim 6

Zhang is said to teach a method using at least two different standard sequences on a single vector for measuring a corresponding number of different target sequences.

Zhang is said also to teach PCR and the generation of complementary sequences in order to determine an absolute copy number and a relative copy number (citing the entire document, but especially Fig. 1). As noted above, Zhang is alleged to teach the construction of a standard curves using a standard sequence and the use of correction factors to arrive at accurate results.

Importantly, the Office admits that, in contrast to the present claims, Zhang does **not** specifically teach the construction of a standard curve as done by the present method in order to determine an absolute CN and a relative CN.

The Office concludes, however, that it would have been obvious to modify the teachings of Zhang by constructing standard curves with measurements of a sequence *complementary* to a standard sequence, since Zhang teaches *complementary* sequences and that measurement biases of different sequences can be rectified by correction factors and proper standard curve construction. The Office's basis for "reasonable expectation of success" is said to be in the *suggestion* by Zhang that the standard curve can be constructed using measurements of a sequence *complementary* to a standard sequence. The "motivation" to do so is allegedly provided by Zhang's teaching of the usefulness of standard curves constructed with standard sequence measurements to measure target sequences. Thus, the Office believes that the claimed invention as a whole is *prima facie* obvious over the cited prior art.

<u>Applicants' Response</u>

Zhang discloses a method of determining relative CN of the mRNA product of a gene in the liver. The relative CN is expressed as number of mRNA copies per ng total RNA. As practiced, and claimed, the present method expresses the relative CN as the concentration of the nucleotide sequence of interest (NucSeqI) divided by the concentration of the nucleotide sequence standard (NucSeqII), both of which are accurately measured using control samples with a known ratio (*i.e.*, the ratio of the concentration of NucSeqI' to the concentration of NucSeqII' (claim 1). Thus, the resulting "relative CN" calculated in accordance with the Zhang method is distinct from the relative CN number determined according to the present claims. Therefore, as an initial matter, the skilled artisan would not look to Zhang.

The Action notes that Zhang **<u>does not</u>** specifically teach the construction of a standard curve with measurements of a sequence *complementary* to a standard sequence in order to determine an absolute copy number and a relative copy number. Applicants wish to correct what appears to be a misunderstanding in this notion (and refer to their remarks above in connection with Office Action Items 9 and 10, and the indefiniteness rejections).

In the present claims, NucSeqI and NucSeqI' "correspond" with one another (defined by the present specification as being homologous). In various dependent claims, NucSeqI and NucSeqI' are the same. This is also true for the relationship between NucSeqII and NucSeqII'. Hence, it is not accurate to state that present invention involves construction of a standard curve based on measurements of a sequence *complementary* to a standard sequence. Again, Applicants believe that the misunderstanding arose from their original language that "NucSeqI corresponds to NucSeqI'," but should now be clear now in light of this explanation.

In addition, the following list describes differences between the Zhang method and the present method, and demonstrates that there is no basis for the position that Zhang suggests or otherwise renders obvious, the current claims.

1. Zhang uses NucSeqI and NucSeqI' in one container. NucSeqI' is the RNA "standard" that is modified from NucSeqI (see Zhang Figure 1), wherein primer 1 and primer 5 will result in a smaller amplification product than the target RNA (because part of the sequence between primer 3 and primer 5 has been deleted during generation of the RNA standard).

2. Zhang describes a competitive RT-PCR, wherein NucSeqI and NucSeqI' in a single container compete for the same primers. The present claims do not involve any such competition for the primer (in a single container). In the present invention, NucSeqI and NucSeqII are determined in one container, while NucSeqI' and NucSeqII' are determined in (one

or two) other, separate containers. While this is not explicitly stated in claim 1, it is implicit and would be abundantly clear to a person skilled in the art for the reasons discussed below. In the present invention NucSeqI and NucSeqII are determined in a single container (see Claim 1, Step (2), "wherein clause" (iii). It is understood and implicit in the method, that NucSeqI' and NucSeqII' are determined in (A) a single container separate from the container in which NucSeqI and NucSeqII are determined or (B) in two other separate containers (see specification at page 10, lines 2-10). Therefore the claimed method can be (and preferably is) practiced so that NucSeqI and NucSeqI' yield the same amplification product (and/or NucSeqII and NucSeqII' yield the same amplification product). Differences in nucleotide sequences may lead to differences in the amplification efficiency. Amplification of NucSeqI and NucSeqI' in a single container, using RT-PCR for example, results in indistinguishable amplification products because RT-PCR discriminates among amplification products based on fluorescent signal, not product length (which is the case in competitive PCR). The skilled artisan will understand and appreciate that, even without express recitation in the claim, the use of the same probe excludes the possibility of amplifying both NucSeqI and NucSeqI' in a single container (and/or both NucSeqII and NucSeqII' in a single container). See specification at page 6 lines 15-27).

Again, Applicants respectfully draw the Examiner's attention to the fact that in the practice of the present invention, accuracy is not dependent on NucSeqI and NucSeqI' being in a single container or separate containers, but rather on using another sequence, <u>NucSeqII, which is detected in the same container as NucSeqI</u> (see Examples). A concentration can be determined using a standard curve generated from a dilution series of NucSeqII' which is situated on a single vector with NucSeqI'.

The present invention therefore provides a solution to two weaknesses of the Zhang method. A disadvantage of Zhang (like that of Douek *et al.*, described at page 4 lines 28-35) is that the DNA extracted from cells must be assumed to be <u>all</u> of the DNA present in the cells (*i.e.*, that no cells escaped lysis and all the DNA originally present in the cells was successfully extracted and isolated). In addition, the method of Zhang is sensitive to differences in amplification efficiency. Because differences in nucleotide sequences may lead to differences in the amplification efficiency, the present claims allow (or, in the dependent claims, require) that NucSeqI and NucSeqI' are the same and/or that NucSeqII and NucSeqII' are the same. This notion is neither disclosed nor suggested in Zhang.

4. Additional "elements" with respect to correction factors that are needed in the Zhang method are related to the amount of total RNA, the PCR efficiency and the amount of

PCR products loaded on to the agarose gel. It is common knowledge to the skilled artisan that any RT-PCR amplification reaction depends on (i) the detection limits of the detection method used, (ii) saturation, if too much of a NucSeq is used at the start of the amplification, and (iii) PCR efficiency. The present claims require detection by fluorescence, which is more sensitive and suffers from fewer limitations than the agarose gel method of Zhang.

5. Zhang. teaches a method of detecting the ratio of a NucSeqI (target RNA) to NucSeqI' (standard RNA), wherein either

(a) the amount of target (=total) RNA is varied while the amount of standard RNA and the number of PCR cycles are kept constant (see page 771; left col., 2nd para) or

(b) the number of PCR cycles is varied while the amounts of target RNA and standard RNA are kept constant (page 771; left col, 5th para). This differs from, and does not suggest, the presently claimed invention, in which

(i) the ratio of a NucSeqI/ NucSeqII is determined, (and not the ratio of NucSeqI/NucSeqI').

(ii) a standard curve is generated using a dilution series of NucSeqI' and NucSeqII" from which the concentration of NucSeqI and NucSeqII, respectively, are determined. Thus, in the amplification, neither the amount of NucSeqI nor the number of cycles is varied.

6. Zhang do not disclose nor suggest using >1 standard RNA on a single vector. For each of the fibrinogen isoforms described in Zhang, <u>individual</u> controls have been generated (page 770; S*ynthesis and Preparation of Oligonucleotides*) (as compared to present claim 3); In fact, Zhang does not disclose or even suggest that the standard RNA be localized on a vector at all. This is so because the Zhang's standard RNA is a PCR product. In contrast, the present claims recite a NucSeqI' (or in dependent claims, >1 different NucSeqI' sequences, in addition to NucSeqII' that are situated on a single vector (which vector, as described, may be linearized).

7. In Zhang, NucSeqI' (standard RNA) is used both as internal control and for calculating the number of copies of target RNA (page 771; right col, 7th para). Since the concentration of standard RNA added to the reaction is known, the number of copies of standard RNA can be determined using Avogadro's number (page 771 left col, para. 7 through right col, 1st para). However, since the determination of standard RNA concentration is rather inaccurate, the copy number so determined will not be very accurate either. The present claims use a second NucSeq (NucSeqII) (and, in dependent claims, the copy number per cell of this sequence is

known). Since NucSeqII is determined using a standard curve which is generated from a dilution series of NucSeqII' (present in a known ratio to NucSeqI'), the accuracy of the claim method is markedly improved over Zhang in a way that is not suggested by, or obvious from, Zhang.

Applicants believe that the Office has not provided a basis as to why a skilled artisan reading Zhang would have included these elements or steps which clearly distinguish the present invention from anything in the Zhang reference. The foregoing remarks emphasize the distinctions of the present invention from Zhang and set forth reasons why the invention as claimed is not obvious over Zhang. Therefore, in view of the foregoing, it would be proper to withdraw the ejections under §103(a).

VI. Additional References of Record

Applicants acknowledge that the following two references were noted by the Examiner as being "of interest" to the instant application but were not applied in any rejections.

D.G. Ginzinger *et al.*. "Measurement of DNA Copy Number at Microsatellite Loci Using Quantitative PCR Analysis", Canc Res 60:5405-5409 (2000)

J. Zhang *et al. et al.*, "Differential priming of RNA templates during cDNA synthesis markedly affects both accuracy and reproducibility of quantitative competitive reverse-transcriptase PCR." *Biochem J. 337*:231-241 (1999), which is said to "teach the design and use of primers for determining a copy number."

VII. CONCLUSION

In view of the amendments to the claims and the foregoing remarks, Applicants believe that they have overcome or mooted the various grounds for rejection. Reconsideration, withdrawal of the rejections and allowance of the amended claims are respectfully requested.

The Examiner is respectfully requested to contact the undersigned at (202) 628-5197 if any clarification is required or if further discussion will assist in continued examination of this application

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